Synthesis and structure–activity relationship of 2,6-disubstituted pyridine derivatives as inhibitors of β-amyloid-42 aggregation

Heiko Kroth a, Nampally Sreenivasachary a, Anne Hamel a, Pascal Benderitter a,b, Yvan Varisco a, Valérie Giriens a, Paolo Paganetti a,c, Wolfgang Froestl a, Andrea Pfeifer a, Andreas Muhs a,e

a AC Immune SA, EPFL Innovation Park, Building B, 1015 Lausanne, Switzerland
b Oncodesign, 20, rue Jean Mazen, 21076 Dijon, France
c AC Immune SA, EPFL Innovation Park, Building B, 1015 Lausanne, Switzerland

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It is assumed that amyloid-β aggregation is a crucial event in the pathogenesis of Alzheimer’s disease. Novel 2,6-disubstituted pyridine derivatives were designed to interact with the β-sheet conformation of Aβ via donor–acceptor–donor hydrogen bond formation. A series of pyridine derivatives were synthesized and tested regarding their potential to inhibit the aggregation of Aβ. The 2,6-diaminopyridine moiety was identified as a key component to inhibit Aβ aggregation. Overall, compounds having three 2,6-disubstituted pyridine units separated by at least one C2- or C3-linker displayed the most potent inhibition of Aβ aggregation.

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Alzheimer’s disease (AD) is the most common age-related neurodegenerative disorder affecting an estimated 36 million patients in 2010. Current treatment options are limited to drugs treating the symptoms and do not slow or reverse disease progression. Thus, a disease modifying drug would be a major therapeutic breakthrough towards treatment of the disease. Although the mechanisms of neurodegeneration in AD are not yet fully discovered, some major pathological signs, such as senile plaques (SP) composed of amyloid-β (Aβ) peptides and neurofibrillary tangles composed of hyperphosphorylated tau protein, are characterized.2,3 SPs consist predominantly of the 40–42 amino acid Aβ peptides, which are derived from the amyloid precursor protein (APP). The formation of Aβ fibrils, via aggregation of soluble Aβ in an antiparallel β-sheet conformation,4 and their deposition into neurotoxic amyloid plaques is considered an initial event in the progression of AD.5 Recent studies on Aβ toxicity suggest that an early stage involves the aggregation of extracellular soluble Aβ peptide into low molecular weight soluble oligomers or high molecular weight prefibrillar intermediates.6–8 A central therapeutic aim in AD is the removal of toxic β-amyloid deposits,9 which can be achieved by secretase inhibitors (inhibition of Aβ production), drugs promoting β-amyloid clearance via active or passive immunotherapy or inhibition of Aβ aggregation and toxicity. Preventing Aβ aggregation is therapeutically attractive because the process is believed to be a purely pathological event and does not interfere with the physiological role of APP.10

We had already prepared small molecule inhibitors of Aβ aggregation based on the connection of two substituted 3-aminopyrazole moieties via a linker to enable donor–acceptor–donor hydrogen bond interactions complementary to that of the β-sheet conformation of Aβ and additional π–π stacking/hydrophobic interactions with amino acid residues of Aβ.11 This previous series of Aβ aggregation inhibitors based on the 3-aminopyrazole moiety, however, displayed low solubility at physiological pH (<10 μM) and low metabolic stability leading to poor bioavailability. The aim of the novel 2,6-disubstituted pyridine series was to maintain potency but to increase solubility as well as metabolic stability. Overall, the novel inhibitors should contain up to 3 hydrogen bond donors and 3 hydrogen bond acceptors. The individual 2,6-disubstituted pyridine moieties should be connected via C1- to C3-linker units to identify the optimal linker length (Fig. 1).

To synthesize the novel Aβ aggregation inhibitors containing two or three 2,6-disubstituted pyridine moieties, it was necessary to prepare suitable building blocks. The synthesis of monomeric 2,6-disubstituted pyridine building blocks is shown in Schemes 1 and 2. The starting material, compound 1, to synthesize compounds 2–9 was prepared as described.12 Building blocks 3, 7
using the same conditions employed for the synthesis of 

Scheme 1. Reagents and conditions: (a) NaH, DMF, CHJ, 0 °C to rt, 16 h, 74%; (b) LDA, THF, –78 °C; (ii) DMF, –78 °C; (iii) HOAc, MeOH, NaBH₄, –78 °C to rt, 35–44%; (c) CH₃SO₂Cl, TEA, DCM, 0 °C to rt, 1 h; (d) NaN₃, DMA, 75 °C, 16 h, 89–95%; (e) TPP, THF, H₂O, rt, 24 h, 82–90%; (f) NBS, AIBN, CCl₄, 100 °C, 5 h, 25%.

and 9 were prepared via 3–5 steps. First, compound 1 was alkylated with methyliodide using sodium hydride. Next compound 2 was treated with lithiumdiisopropylamine (LDA) at –78 °C, followed by the addition of dimethylformamide to obtain the C1-elongated aldehyde intermediate. Reduction with sodium tetrahydroborohydride at –78 °C in the presence of acetic acid and methanol yielded the corresponding alcohol. Activation of the alcohol with methanesulfonylchloride followed by displacement with sodium azide under neutral conditions yielded the azide under Staudinger conditions afforded the amine building block 11. Staudinger reduction as described for 11 to afford the dimeric building block 14 containing a C3-linker. The corresponding building block 16 containing a C1-linker was prepared from 15 using commercially available Boc-2-amino-pyridine and the alkylation conditions employed for the preparation of 2. Starting material 15 was synthesized as described. Attempts to use the methanesulfonate derivative of 3 under the basic conditions described for the preparation of 16 resulted in the formation of the corresponding vinylpyridine derivative of 3.

The dimeric building blocks 17 and 19 (Scheme 3) were both prepared from compound 7. Alkylation of 7 with 8 followed by Staudinger reduction as described for 5 afforded the dimeric building block 17 containing a C1- and a C2-linker. Alkylation of compound 7 with 15 afforded compound 18. Removal of the bromosubstituent and reduction of the azide was accomplished by catalytic hydrogenation with palladium on carbon to afford the dimeric building block 19 containing a C1- and a C2-linker.

The starting material 20 for the preparation of dimeric building blocks 23 and 25 (Scheme 4) was prepared as described. Protection of the alcohol with the triisopropylsilyl (TIPS) moiety gave 21. Compound 21 was then reacted with 5 using tris(dibenzylidene-nacetonetetrapalladium(0)(P₃dba)₂, 2,2′-Bis-(diphenylphosphinoo)-1,1′-naphtalene (BINAP) and sodium tert.-butoxide under Buchwald conditions. The use of 10 mol % palladium catalyst and a short reaction time (45 min) were critical for good conversion and yield. Extended reaction times lead to significant decomposition of 22 in the presence of the strong base sodium tert-butoxide.

Figure 1. Possible hydrogen bond interactions of 3-aminopyrazole or 2,6-disubstituted pyridine derivatives with the β-sheet conformation of Aβ and inhibitor design.

Inhibitors containing 2 pyridines

Inhibitors containing 3 pyridines

Scheme 2. Reagents and conditions: (a) (i) allylalcohol, 9-BBN, THF 0 °C, 4 h; (ii) Pd[P(Ph)₃]₄, THF, NaOH, DMA, 95 °C, 90 min, 79%; (b) phthalimide, TPP, THF, DEAD, rt, 16 h, 86%; (c) N₂H₄ × H₂O, MeOH, rt, 16 h, 60%.

Inhibitors containing 2 pyridines

Inhibitors containing 3 pyridines

Figure 1. Possible hydrogen bond interactions of 3-aminopyrazole or 2,6-disubstituted pyridine derivatives with the β-sheet conformation of Aβ and inhibitor design.
No formation of dimeric coupling products was observed under the reaction conditions. The free NH-group in 22 was Boc-protected at elevated temperature. The best results were obtained by dissolving 22 and di-tert-butyl dicarbonate (Boc₂O) in dichloromethane and evaporation of the solvent followed by heating of the oily reaction mixture. After cleavage of the TIPS-group with potassium hydrogencarbonate, the free OH-group was converted via 3 additional steps to the amine as described for 5 to afford the dimeric building block 23 containing two C₂-linkers. The synthesis of the dimeric building block 25 containing two C₂-linkers was performed as described for 23 except that commercially available 2-(2-aminoethyl)-pyridine was used for the Pd-coupling reaction.

The preparation of the dimeric building blocks 28 and 30 via 2–6 steps is shown in Scheme 5. Commercially available 2,6-dibromopyridine was converted to 26 via Suzuki coupling with the reaction product of 9-BBN and allylalcohol followed by TIPS-protection of the coupling product as described for 11. Compound 26 was then coupled under Buchwald conditions with 3-(pyridin-2-yl)propan-2-amine, which was prepared as described.¹⁸

Subsequent Boc-protection and cleavage of the TIPS-group as described for 23 afforded 27. Conversion of the OH-group to the corresponding amine was performed as described for 12 to obtain the dimeric building block 28 containing two C₃-linkers. Treatment of 2,6-dibromopyridine with 2(2-aminoethyl)pyridine at elevated temperature in the presence of potassium hydrogencarbonate afforded 29, which was Boc-protected as described for 23 to obtain the corresponding dimeric building block 30 containing a C₂-linker.

The synthesis of Aβ aggregation inhibitors containing either two or three 2,6-disubstituted pyridines and different linkers is shown in Schemes 6–8. The inhibitors 31–34 (Scheme 6) were prepared by using building blocks 5, 9, 12, 14, and 16. First, the building blocks were combined via Buchwald coupling¹⁹ at 85 °C for 45 min to yield the corresponding Boc-protected inhibitors. Under the reaction conditions no di-substituted coupling products were observed. Cleavage of the Boc-group under acidic conditions afforded the desired inhibitors as hygroscopic HCl-salts. The best way to obtain the inhibitors was to remove the organic solvents by syringe and to dissolve the precipitated HCl-salt in water. Lyophilization of the aqueous solution then afforded the Aβ aggregation inhibitors as pale yellow/orange powders. Thus, 31 was prepared from 9 and 16; 32 was prepared from 5 and 16; 33 was

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Scheme 3. Reagents and conditions: (a) (i) 9-BBN, THF 0 °C, 4 h; (ii) THF, NaOH, Pd [P(Ph)₃]₄, DMA, 2,6-dibromopyridine, 95 °C, 90 min, 69%; (b) NaH, DMF, Boc-2-amino-pyridine, 65 °C, 3 h, 82%; (c) NaH, DMF, Boc, 60 °C, 2 h, 72%; (d) TPP, THF, H₂O, rt, 24 h, 68%; (e) NaH, DMF, 15, 60 °C, 2 h, 82%; (f) H₂, Pd/C, EOH, TEA, rt, 90%.
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Scheme 4. Reagents and conditions: (a) TIPS-Cl, imidazole, DMF, rt, 16 h, 87%; (b) Pd₂(dba)₃, BINAP, NaOtBu, toluene, 5, 85 °C, 45 min, 78%; (c) Pd₂(dba)₃, BINAP, NaOtBu, toluene, 2-(2-aminoethyl)-pyridine, 85 °C, 45 min, 81%; (d) Boc₂O, 70 °C, 16 h, 93%; (e) BTAf, THF, CH₃CN, rt, 16 h, 88–92%; (f) CH₅SO₂Cl, TEA, DCM, 0 °C to rt, 1 h, 85–90%; (g) NaN₃, DMA, 75 °C, 16 h, 65–92%; (h) TPP, THF, H₂O, rt, 24 h, 85–93%.
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Scheme 5. Reagents and conditions: (a) allylalcohol, 9-BBN, THF 0 °C, 4 h; (ii) Pd [P(Ph)₃]₄, THF, NaOH, DMA, 95 °C, 90 min, 48%; (b) TIPS-Cl, imidazole, DMF, rt, 16 h, 79%; (c) Pd₂(dba)₃, BINAP, NaOtBu, 3-(pyridin-2-yl)propan-2-amine, toluene, 85 °C, 45 min, 83%; (d) Boc₂O, 70 °C, 16 h, 53–92%; (e) BTAf, THF, CH₃CN, rt, 16 h, 94%; (f) (i) TPP, phthalimide, THF, DEAD, rt, 16 h; (ii) N₂H₄, MeOH, rt, 16 h, 62%; (g) KHCO₃, DMA, 2-(2-aminoethyl)pyridine, 110 °C, 5 h, 21%.
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Scheme 6. Reagents and conditions: (a) Pd₂(dba)₃, BINAP, NaOtBu, toluene, 85 °C, 45 min, 50–82%; (b) 2 M HCl, Et₂O, CHCl₃, rt, 16 h, 69–85%.
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prepared from 5 and 14, and 34 was prepared from 12 and 14 (Scheme 6).

The inhibitors 35–38 were prepared from 17 and 23 via coupling with 2-bromopyridine under Buchwald conditions (Scheme 7). Congruent to literature data, the use of 2-bromopyridine under Buchwald conditions of 17 and 2-bromopyridine, whereas the Boc-protected derivatives 35 or 37 or 38. Thus, the Boc-protected derivatives of 35 and 37 were obtained from 17 and 2-bromopyridine, whereas the Boc-protected derivatives of 36 and 38 were obtained from 23 and 2-bromopyridine (Scheme 7).

After acidic cleavage of the Boc-protection groups, the desired inhibitors 35–38 were obtained as hygroscopic solids.

The Boc-protected inhibitors 39–41 were prepared via palladium catalyzed coupling of 19, 25 and 28 with 10 at 110 °C for 45 min (Scheme 8). Since the coupling resulted in the formation of a 2,6-diaminopyridine moiety, a higher reaction temperature was required. The Boc-protected dimeric inhibitors 42 and 43 were prepared in the same manner by coupling 2-(2-aminoethyl)pyridine and 5 with 10. The Boc-protected inhibitor 44 was prepared by coupling of 30 with 5. Unlike the reactions with 2-bromopyridine, no di-substituted palladium coupling products were observed. The synthesis of inhibitors 39–44 was completed by cleavage of the Boc-protection group with acid (Scheme 8).

The inhibition of Aβ aggregation in the presence of 31–44 was determined using a thioflavin T (ThT) fluorescence assay and crude Aβ1-42 peptide film at 33 μM as described. First, 31–38 were screened and their capability to reduce the ThT fluorescence signal of the control reaction (Aβ1-42 aggregation in DMSO) at 330 μM was determined (Fig. 2). The data in Figure 2 showed that 31 containing two C1-linkers, 32 having a C1-linker to the left and C2-linker to the right of the central pyridine, and 35 having a C2-linker to the left and a C1-linker to the right of the central pyridine displayed rather low inhibition of Aβ1-42 aggregation properties (>60% of the control value). Thus, compounds with one or two short C1-linkers most likely could not form strong donor–acceptor–donor interactions with the β-sheet conformation of Aβ1-42. In contrast, 37 containing a bis-pyridyl moiety, 33 having a C3-linker to the left and a C2-linker to the right, and 34 containing two C3-linkers displayed better inhibition of Aβ1-42 aggregation properties (23–29% of the control value). This indicated a preference for compounds with either longer and more flexible linkers (33, 34) or the capability to form additional π–π interactions (37). For all compounds containing three 2-aminopyridine moieties separated by different linkers, 36 and 38 displayed the best inhibition of Aβ1-42 aggregation properties (16% of the control value). In contrast to 35, compound 36 containing two C2-linkers displayed a similar potency as 38 containing a bis-pyridyl moiety. This suggests that proper hydrogen bond donor interactions of each of the three 2-aminopyridyl moieties in 36 have a similar effect than additional π–π interactions for 37 and 38. The distance between donor and acceptor should be ideally in the range of 3.5–4.0 Å and the distance between acceptor and donor should be 2.6–2.9 Å. Superiority of the C2-linker in this series was also evident from the stronger inhibition of Aβ1-42 aggregation of 38 compared to 37.

In order to evaluate the effect of different distribution patterns of the three hydrogen bond donors and acceptors, compounds 39–44 were prepared and their inhibition of Aβ1-42 aggregation properties tested (Fig. 2). Taken the results for 31–38 into account, the C2-linker was preferentially incorporated into compounds 39–44. In contrast to 31–38, each of compounds 39–44 contained a 2,6-diaminopyridine moiety. The 2,6-diaminopyridine moiety in 39–43 is located at a terminal right position, whereas in 44 the

![Scheme 7](image)

**Scheme 7.** Reagents and conditions: (a) Pd(dba)$_2$, BINAP, NaOtBu, toluene, 85 °C, 45 min, 7–22%; (b) 2 M HCl, Et$_2$O, CHCl$_3$, rt, 16 h, 57–97%.

![Scheme 8](image)

**Scheme 8.** Reagents and conditions: (a) Pd(dba)$_2$, BINAP, NaOtBu, toluene, 10, 110 °C, 45 min, 22–89%; (b) 2 M HCl, Et$_2$O, CHCl$_3$, rt, 16 h, 72–84%. (c) Pd(dba)$_2$, BINAP, NaOtBu, toluene, 5, 110 °C, 45 min, 94%.

![Figure 2](image)

**Figure 2.** In vitro screening assays using Aβ1-42 peptide film. The concentration of Aβ1-42 peptide film was 33 μM. The test concentration for compounds 31–44 was 330 μM and the incubation time was 24 h. Data are expressed as percentage (mean ± S.D.) of control conditions: Aβ1-42 aggregation with DMSO only. Freshly prepared Aβ1-42 peptide film (4 μg) was analyzed by SDS-PAGE to confirm the presence of oligomeric Aβ1-42 present (a, molecular weight marker; b, Aβ1-42 peptide film).
position is central. To get an idea on how many hydrogen donors and acceptors are required for efficient inhibition of \( A_b^{1–42} \) aggregation, the truncated compounds 42 and 43 were prepared as well.

The results for 39–41 and 44 clearly showed that all compounds containing the 2,6-diaminopyridine unit displayed efficient inhibition of \( A_b^{1–42} \) aggregation (12–16% of the control value). The position of the 2,6-diaminopyridine moiety, i.e. terminal right (39–41), or central (44), had no significant impact on their inhibition of \( A_b^{1–42} \) aggregation properties in the screening assay. The same applied for the use of C2-linkers (40) or C3-linkers (41). Interestingly, compound 39 was equally potent (14% of the control value) when compared to 40 and 41. This was in sharp contrast to compound 32 (53% of the control value), although the linker composition and number of hydrogen bond donors and acceptors were identical. This suggests the 2,6-diaminopyridine moiety is a preferred unit to enable donor–acceptor interactions with the \( \beta \)-sheet conformation of \( A_b^{1–42} \). Further evidence came from the “truncated” inhibitors 42 and 43 containing a 2,6-diaminopyridine unit. Compound 42 displayed a higher inhibition of \( A_b^{1–42} \) aggregation (36% of the control value) with just 2 hydrogen bond donors and acceptors when compared to 31, 32, and 35 having 3 hydrogen bond donors and acceptors (Fig. 2). Compound 43 having 3 hydrogen bond donors and 2 hydrogen bond acceptors was equally potent as 33 and 34, indicating the importance of hydrogen bond donors for inhibition of \( A_b^{1–42} \) aggregation.

In order to better compare inhibitors displaying >80% inhibition of the control value in the ThT-screening assay, the IC\(_{50}\) for 36, 38, 39, 40, 41, and 44 was determined using the ThT fluorescence assay. To compare our inhibitors with \( A_b \) aggregation inhibitors from literature, the IC\(_{50}\) of Congo Red and Curcumin were determined as well (Fig. 3).

The IC\(_{50}\) determination for 36, 38, 39, 40, 41, and 44 clearly showed that compounds containing a 2,6-diaminopyridine moiety (39–44) displayed a better inhibition of \( A_b^{1–42} \) aggregation (13–35 µM) when compared to 36 and 38 (240–280 µM) having a 2-aminopyridine moiety (Fig. 3). Compound 44 bearing a central 2,6-diaminopyridine was slightly less active (35 µM) than 39, 40, and 41 containing a terminal right 2,6-diaminopyridine moiety. Compounds 39–41 displayed comparable \( A_b^{1–42} \) aggregation properties with IC\(_{50}\) values of 13 µM, 25 µM, and 17 µM, respectively.

The control compound Curcumin inhibited \( A_b^{1–42} \) aggregation in the same range (9 µM). Congo Red was the most potent compound (1.3 µM) in this assay.

The aqueous solubility of selected compounds 31, 40, 41, 43, and 44 at physiological pH (PBS, pH 7.4) were determined as 18 µM, >200 µM, 103 µM, 195 µM and >200 µM, respectively. Compared to the previous 3-aminopyrazole class of \( A_b^{1–42} \) aggregation inhibitors\(^{11}\), the introduction of 2,6-disubstituted pyridines moieties allowed to improve the aqueous solubility of \( A_b^{1–42} \) aggregation inhibitors from <10 µM (3-aminopyrazoles) to >200 µM (40 and 44) while maintaining similar potency.

Overall, compound 40 appeared to be a good compromise between inhibition of \( A_b^{1–42} \) aggregation and high aqueous solubility and was further profiled in vitro. Though the metabolic stability of 40 in human liver S9 fraction was low (\( t_{1/2} = 10 \text{ min}; 0.07 \text{ mL/min/mg} \)), 40 displayed very high permeability (\( P_{eff} = 49.2 \times 10^{-6} \text{ cm/s} \)), was not a P-gp substrate (Efflux ratio = 0.78) and displayed a favorable Log D (2.96). Thus, the pharmacokinetic properties of compound 40 were tested in male Swiss mice (Fig. 4, Table 1).\(^{23}\)\(^{24}\) After intravenous application at 2 mg/kg, compound 40 rapidly entered the brain (1845 ng/g after 5 min) in line with the excellent in vitro permeability. However, the poor metabolic stability was most likely the reason for the rapid clearance of 40 from the brain.

In summary, we have identified the 2,6-diaminopyridine moiety as a key component in the design of potent inhibitors of \( A_b^{1–42} \) aggregation with good aqueous solubility. The most balanced profile between inhibition of \( A_b^{1–42} \) aggregation and aqueous solubility was achieved by separating the 2,6-diaminopyridine unit via a C2-linker from the adjacent 2,6-disubstituted pyridine(s). At least three 2,6-disubstituted pyridine moieties containing 3 hydrogen bond donors and 3 hydrogen bond acceptors in the correct arrangement, i.e. compounds 39–41, 44, were required for the most potent inhibition of \( A_b \) aggregation. Further efforts describing additional compounds with improved metabolic stability and pharmacokinetic properties are in progress.

### Table 1

<table>
<thead>
<tr>
<th>Compounds</th>
<th>40 (plasma)(^{a})</th>
<th>40 (brain)(^{b})</th>
</tr>
</thead>
<tbody>
<tr>
<td>( t_{1/2} ) (h)</td>
<td>0.63 ± 0.18</td>
<td>5.23 ± 2.71</td>
</tr>
<tr>
<td>AUC (ng/mL * h)</td>
<td>150 ± 13</td>
<td>863 ± 195</td>
</tr>
<tr>
<td>( V_{z} ) (L/Kg)</td>
<td>11.99 ± 3.24</td>
<td>18.94 ± 12.93</td>
</tr>
<tr>
<td>( C_{max} ) (ng/mL)</td>
<td>308 ± 7</td>
<td>1845 ± 484</td>
</tr>
<tr>
<td>( B/P )</td>
<td>5.98 ± 1.51</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) Administration at 2 mg/kg i.v.
\(^{b}\) iv formulation (100% DMSO).
\(^{c}\) B/P = brain to plasma ratio, 5 min post i.v. dosing.
Supplementary data

Supplementary (experimental procedures for the preparation of compounds 2–9, 11, 12, 14, 16, 17–19, 21–25, 26–44, in vitro fluorescence assay) data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.05.040.

References and notes

24. The pharmacokinetic study of 40 was conducted by Eurofins ADME Bioanlyses (France) using 42 male Swiss mice around 5 weeks old. The animals were fed on pellets. The pellets and tap water were given “ad libitum”. During the experiments, the animals were fasted at least 8 hours before the dosing. The administered dose of compound 40 was 2 mg/kg at a concentration of 2 mg/mL using DMSO as vehicle. Animals were anaesthetised with isoflurane® using an anaesthetic system for administrations, at intermediary blood sampling times and at sacrifice.